

**First description of larval and juvenile stages
of *Rhacophorus maximus* Günther, 1859 “1858”
(Anura: Rhacophoridae) from Vietnam**

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First description of larval and juvenile stages of *Rhacophorus maximus* Günther, 1859 “1858” (Anura: Rhacophoridae) from Vietnam. - We describe the tadpole morphology of the Nepal flying frog, *Rhacophorus maximus* Günther, 1859 “1858”, based on specimens originating from the lowland evergreen forest of the Tay Yen Tu Nature Reserve in the Bac Giang Province in northern Vietnam. The description is based on exotrophic larvae of ORTON's type IV: lentic, benthic, nektonic in developmental stages 33-41, which were bred at the IEBR Amphibian Breeding Station in Hanoi and identified by DNA barcoding. DNA sequences of the mitochondrial 16S rRNA gene obtained from a tadpole voucher in developmental stage 35 used for the description had less than 0.18% sequence divergence to those of a sympatric adult frog, making the identification unambiguous. Tadpoles of *R. maximus* are of more generalized morphology with a labial tooth row formula of 5(2-5)/3(1). In addition, based on (hand-) reared froglets and by identifying wild-caught subadults through DNA barcoding, we provide first data on colour pattern change from metamorphosed to adult stages.

Keywords: Rhacophoridae - *Rhacophorus maximus* - DNA barcoding - tadpole - morphology - development - colour pattern change - Vietnam.

INTRODUCTION

The investigation of amphibian diversity in Vietnam strongly increased in recent decades. Only in the past six years, 26 Vietnamese anuran species have been discovered (Nguyen *et al.*, 2009). However, in contrast to the increasing richness of amphibian species, many of them are only known from a few specimens preserved in

museums and virtually nothing is known about the natural history of adults and in particular of their larval stages. The knowledge of larval adaptations is inadequate or often completely lacking up to this date, but it is a crucial prerequisite for adequate amphibian conservation measures. This deficiency is due to the fact that detailed morphological larval descriptions are still lacking for many species, e.g. the tree frog *Rhacophorus maximus*.

R. maximus was described by Günther (1859 “1858”) from the type locality “Nepal”. The Nepal flying frog is generally characterized by its large size, bright green dorsal colouration in combination with a narrow white flank stripe, and complete webbing between fingers and toes (Anders & Rai, 2002). The crepuscular species inhabits the canopy of lowland to submontane moist evergreen forest. Its distribution ranges from North-Eastern India to Southern China, Western Thailand and Northern Bangladesh (Frost, 2009). Orlov *et al.* (2008) mentioned the species’ presence in Vietnam, but did not provide specimen-based, specific locality records. Only recently, Nguyen *et al.* (2008) provided a first detailed record of *R. maximus* from Vietnam, namely from the Tay Yen Tu Nature Reserve in the Bac Giang Province (Figs 1-2), which is part of the largest continuous area of lowland evergreen forest in north-eastern Vietnam (Birdlife International, 2004). As this type of forest has been cut down in most other areas of north-eastern Vietnam, this region is expected to house a unique biodiversity that is no longer found elsewhere in the country. According to the IUCN Red List of threatened species the status of *R. maximus* is least concern and the population trend is decreasing.

The knowledge of the larval stages of *Rhacophorus maximus* is poor. Sinha *et al.* (2001) conducted food spectrum analyses based on tadpoles of *R. maximus* from India, and Khongwir *et al.* (2003) studied metamorphosis and the development of the mouth region. Drawings of tadpoles from Nepal in an obviously early developmental stage were published by Anders & Rai (2002), who also dealt with the advertisement call of the species. However, no comprehensive descriptions on the larval morphology of *R. maximus* are available.

Thus, the object of this paper is to provide a first detailed description of the larval morphology of *R. maximus* from Vietnam. This description is based on larvae originating from adult frogs from the Tay Yen Tu Nature Reserve. In addition, based on froglets (reared by us) together with wild-caught subadults from the Tay Yen Tu Nature Reserve partly identified by DNA barcoding, we also describe for the first time colour pattern change from metamorphosed individuals towards adult stages.

MATERIAL AND METHODS

A series of larvae, juveniles, subadults, and adults of *Rhacophorus maximus* from the Tay Yen Tu Nature Reserve, Bac Giang Province, northern Vietnam were used for the following descriptions. Adults identified by size and gonad development were collected in the field; larval, juvenile and subadult stages derived from successful breeding of *R. maximus* from Tay Yen Tu at the Amphibian Breeding Station of the Institute of Ecology and Biological Resources in Hanoi. We used fresh dead specimens for the morphological descriptions, fixed in 40-70% ethanol, preserved in 70% ethanol and subsequently deposited in the following museums: IEBR = Institute of Ecology



FIG. 1

Location (large dot) of the Tay Yen Tu Nature Reserve, Bac Giang Province, North-Eastern Vietnam, the so far only known record of *Rhacophorus maximus* from Vietnam.

and Biological Resources, Hanoi, Vietnam; MHNG = Muséum d'histoire naturelle, Geneva, Switzerland; VNMN = Vietnam National Museum of Nature, Hanoi, Vietnam; ZFMK = Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

Specific vouchers were used as follows: adult: IEBR 3653; subadults: IEBR A.0940, MHNG 2721.50, VNMN 1211, ZFMK 89786; juveniles: MHNG 2721.51, VNMN 1212, ZFMK 89787-89788; froglets: IEBR A.0941, MHNG 2721.52, ZFMK 89790; tadpoles: IEBR A.0942, MHNG 2721.53, VNMN 1214, ZFMK 89791-89792. The colour pattern was studied in juvenile, subadult, and adult stages currently kept at the Amphibian Breeding Station at Hanoi, at the Aquarium of the Cologne Zoo, and on wild-caught specimens from Tay Yen Tu, which were released subsequently.

Several studies have demonstrated that DNA barcoding, especially when using the mitochondrial 16S rRNA gene, is a reliable identification tool in amphibian taxonomy (e.g., Vences *et al.*, 2005; Bwong *et al.*, 2009). We used this method to unambiguously assign specific vouchers to *R. maximus*. For the assignment of the tadpoles of *R. maximus*, molecular data were obtained for a larva in Gosner stage 35 (IEBR A.0942; GenBank accession number HM448032), which also served for the larval description and illustration (with a partly removed tail muscle tissue due to the taken tissue sample). We compared the resulting sequence with homologous DNA fragments of a syntopic, adult *R. maximus* (IEBR 3653; GenBank accession number HM448031; see also Nguyen *et al.* 2008). We also compared DNA fragments of this adult specimen with a subadult specimen collected in Tay Yen Tu (IEBR A.0940; GenBank accession number HM448030), having a divergent colour pattern, so far not recorded for *R. maximus*. DNA was extracted using the peqGold Tissue DNA Mini Kit (PEQLAB Biotechnologie GmbH). The primers 16sar-L (light chain; 5' – CGC CTG TTT ATC AAA AAC AT – 3') and 16sbr-H (heavy chain; 5' – CCG GTC TGA ACT CAG ATC ACG T – 3') of Palumbi *et al.* (1991) were used to amplify a section of the mitochondrial 16S ribosomal RNA gene (569 bp). PCR cycling procedure followed Schmitz *et al.* (2005). PCR products were purified using QIAquick purification kits (Qiagen). Sequences (including complimentary strands for ensuring the accuracy of the sequences) were obtained using an automatic sequencer (ABI 377). Sequences were examined and aligned manually using the original chromatograph data in the program BioEdit (Hall, 1999). The same program was used to calculate the direct pairwise similarities for the resulting sequences.

The terminology for adult, subadult, and juvenile stages followed Manthey & Grossmann (1997) and Glaw & Vences (1994). The tadpole terminology, morphometrics and abbreviations (partly modified) followed Altig & McDiarmid (1999) and Grosjean (2005), the labial tooth row formula (LTRF) was determined according to Altig & McDiarmid (1999), and the keratodont row formula (KRF) according to Dubois (1995); for general larval types see Orton (1953). Determination of larval stages was set out according to Gosner (1960). Colouration in life of all specimens was described by means of photographs.

Abbreviations used for morphological descriptions are as follows. *Adults, subadults, juveniles*: SVL = snout-vent length; HW = maximum head width; HL = head length, from tip of snout to end of lower jaw; EN = distance between anterior angle of eye to centre of nostril; NS = distance between centre of nostril to tip of snout; ED =



FIG. 2

Habitat of *Rhacophorus maximus* in the Tay Yen Tu Nature Reserve, Bac Giang Province, north-eastern Vietnam. Photograph by T. Ziegler.

horizontal diameter of eye; TD = horizontal diameter of tympanum; FLL = length of fore limb, up to the tip of the longest (third) finger; HLL = length of the extended hind limb from cloaca to tip of longest toe; FL = length of foot, exclusive of tarsus, from base of inner metatarsal tubercle up to tip of longest (fourth) toe; IMT = length of inner metatarsal tubercle.

Larvae: BH = body height (midpoint of body); BL = body length; BW = body width (midpoint of body); ED = maximum diameter of eye (horizontal); IP = interpupilar distance (from midpoint of eyes); IND = internarial distance; NPD = narpupilar distance; RND = rostro-narial distance (from tip of snout); SP = snout-pupil distance; ODW = oral disc width; NK = number of keratodonts (per 0.5 mm of the A3 keratodont row); NP = number of papillae around mouth; SS = snout-spiracle distance (to opening of spiracle); TL = total length; TAL = tail length (from beginning of ventral tube); TMH = height of tail musculature at base; TMW = width of tail musculature at base; UF = height of upper tail fin (midpoint of tail); LF = height of lower tail fin (midpoint of tail); MTH = total tail height (midpoint of tail). All measurements are approximate values and were taken in millimetres (mm) with a digital calliper gauge.

RESULTS

LARVAL IDENTIFICATION OF *RHACOPHORUS MAXIMUS* FROM VIETNAM

An unambiguous assignment of a tadpole in stage 35 (IEBR A.0942), which served for the description of the external morphology, to the species *R. maximus* was guaranteed by its negligible 16S sequence divergence (0.18%, corresponding to only single base pair difference) to a sympatric, adult specimen (IEBR 3653).

The adult male IEBR 3653 largely agreed with the descriptions of *Rhacophorus maximus* provided by Günther (1859 "1858"), Boulenger (1890), Bourret (1942), Fei (1999), Yang *et al.* (1991), Anders & Rai (2002), and Nguyen *et al.* (2008): dorsum in life is uniformly green and the venter is fawn; dorsum in preservative is blue to violet and the venter is light brown; in life as well as in preservative a narrow white stripe runs along the flanks; nares are equidistant from the eye and the tip of snout; supratympanic fold is distinct; fingers and toes are completely webbed; vomerine teeth are present, as are the vocal sacs; the interorbital distance is distinctly wider than the upper eyelid; the metacarpal tubercle is especially well developed in males; SVL of IEBR 3653 is 104.4 mm (SVL of the type specimen is 92.9 mm according to Günther, 1859 "1858", the maximum SVL is 114.3 mm according to Boulenger, 1890); FLL of IEBR 3653 is 57.5 mm, and HLL is 141.0 mm.

TADPOLE DESCRIPTION

Colour pattern in preservative: Body colour is yellow to fawn, dorsally covered with stellate, dense grey to brown pigments, with pigmentation strongest above the nares. The body sides and the venter are only slightly pigmented. Venter is slightly transparent and white to fawn at the region of the intestinal coils. Hind limbs are yellow to white, dorsally covered with dark grey to black pigments. The tail musculature is yellowish-cream with grey pigmentation fading toward the tip. The V-shaped myosepta, the base of the upper fin at the first half of the tail, and the very tip of the tail are much more densely pigmented. The upper and lower tail fins are whitish-cream, slightly transparent with regular grey nodular pigments.

Colouration in life: Body colour is yellow to white, slightly transparent and blotched with golden pigments. Dark greyish pigments on the back are strongest at the nares, above the nostrils and eyes. The intestinal coil is well visible laterally and ventrally. The body venter is blotched with silver pigments. The upper and lower tail fins are transparent. The tail is marbled with dark grey and irregular golden pigments. The whitish yellow tail musculature colouration fades towards the tip (Fig. 3B).

Description in dorsal view: The body shape is oval-elongated (body width 0.51 of body length) with a pointed to angled snout. Eyes are of moderate size (maximum diameter of eye 0.12 of body length), dorsolaterally positioned and directed at the end of the first third of the body (snout-pupilar distance is 0.29 of body length). The interpupilar distance is 0.63 of the body width. Nares are slightly kidney-shaped, anterio-dorsally positioned and laterally directed (rostro-narial distance is 0.64 of nario-pupilar distance). The internarial distance is 0.55 of the interpupilar distance. Spiracle is sinistral and visible. The tadpole has a well developed lateral line organ. On both sides of the head, a line is running from the tip of snout laterally to below the eye, a second line is running from the tip of snout dorsally passing the nare at the inner side and ending dorsally with the eye. The bulge of the oral disc is visible. The tail musculature is of moderate size (width of tail musculature at base is 0.35 of body width) (Fig. 4).

Description in lateral view: Body is slightly depressed (body height is 0.80 of body width). Spiracle is sinistral, laterally positioned at the end of the second third of body (distance of snout tip to opening of spiracle is 0.67 of the body length). The spiracle opening is relatively large, rounded, and directed dorsally. Spiracle is attached to the body wall. The lateral line organ is visible and stretches from below the snout to



FIG. 3

Rhacophorus maximus: (A) Adult, in amplexus, in life in the Tay Yen Tu Nature Reserve, Bac Giang Province, north-eastern Vietnam. (B) Tadpole and (C) Froglet in life at the Amphibian Breeding Station in Hanoi, Vietnam. (D) Juvenile in the terrarium at the Cologne Zoo. Photographs by T. Q. Nguyen (A) and T. Ziegler (B-D).

behind the eyes. The lower lip has marginal papillae sticking out ventrally at the anterior body. The tail length is 1.99 of the body length. The tail musculature is remarkably developed (height of tail musculature at base is 0.64 of body height and 0.61 of maximum tail height) and is more or less equal in height until the second third of the tail before gradually tapering to the tail tip. The upper fin originates at the end of body, it is gradually elevated until midpoint of the tail before tapering until tip of tail (height of upper fin is 0.27 of maximum tail height). The lower tail fin height is constant from base to tip and it is 0.95 of the height of the upper fin at midpoint. Vent tube of IEBR A.0942 is damaged and hence could not be used for description. Therefore, description of vent tube is based on the specimen MHNG 2721.53. It starts ventromedially and fades to the right side in dorsal view, with the opening being remarkably large and elongated. The inner wall is adnated to the lower fin (Fig. 4).

Oral disc: Oral disc is positioned anteroventrally and laterally slightly emarginated (oral disc width is 0.41 of body width). It is framed by marginal, rounded papillae, except for a large medial gap at the upper labium and a very small medial gap at the lower labium. All papillae are of moderate size and are white to transparent. Papillae of the upper labium and medial region show pigmentation whereas papillae of

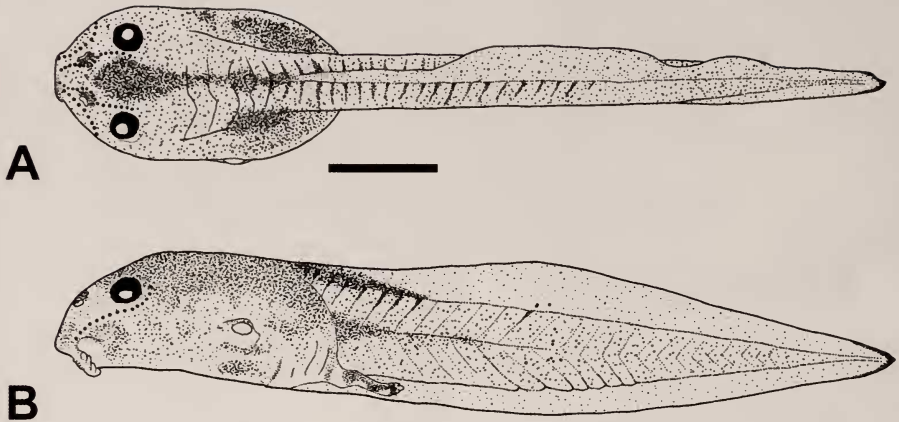


FIG. 4

Drawings of the preserved tadpole of *Rhacophorus maximus* (IEBR A.0942) at Gosner stage 35 (scale bar = 5 mm). (A) dorsal view. (B) lateral view. Drawings by M. J. Wildenhues.

the lower labium lack any pigmentation. The lower labium has one row of submarginal papillae on the right side and two rows of submarginal papillae on the left side. Three separated submarginal papillae are situated left from ventral view. The upper labium has 4-5 submarginal papillae on each side. The oral disc shows 106 papillae in total. The LTRF is 5(2-5)/3(1) and the KRF is 1:4+4/1+1:2 with 35 keratodonts per 0.5 mm of keratodont row A3. Jaw sheaths are dark brown and serrated, except for the lateral endings of the upper jaw sheath. The upper jaw sheath is curved and with long appendices. The lower jaw sheath is V-shaped (Fig. 5).

The specimens MHNG 2721.53, VNMN 1214, and ZFMK 89791-89792, largely correspond to this description (see Table 1 for specific measurements), as do other tadpoles that were only photographed at the Amphibian Station, except for slight variation in the oral disc region (LTRF, KRF, NK, and NP).

DESCRIPTION OF JUVENILE AND SUBADULT STAGES OF *RHACOPHORUS MAXIMUS* FROM VIETNAM

Freshly metamorphosed froglets ($n = 3$; SVL 16.8-19.0 mm): The following description is based on specimen IEBR A.0941 (SVL 19 mm) in stage 46.

In preservative, the dorsum is dark grey to dark brown, with black posterior. The ventral side is fawn to yellow with single pigments on the lower jaw. The dorsal surface of fore and hind limbs is covered with grey to brown pigments and the venter is fawn to yellow. The inner sides of feet and hands are slightly dark pigmented. Colouration of the back fades to colouration of the belly.

In life, back is green with somewhat violet pigmentation, more distinct on the lateral side of the tail stub, around the nares, and above the tympanum. The green region fades to fawn before fading to white on the venter. Fore limbs and hind limbs

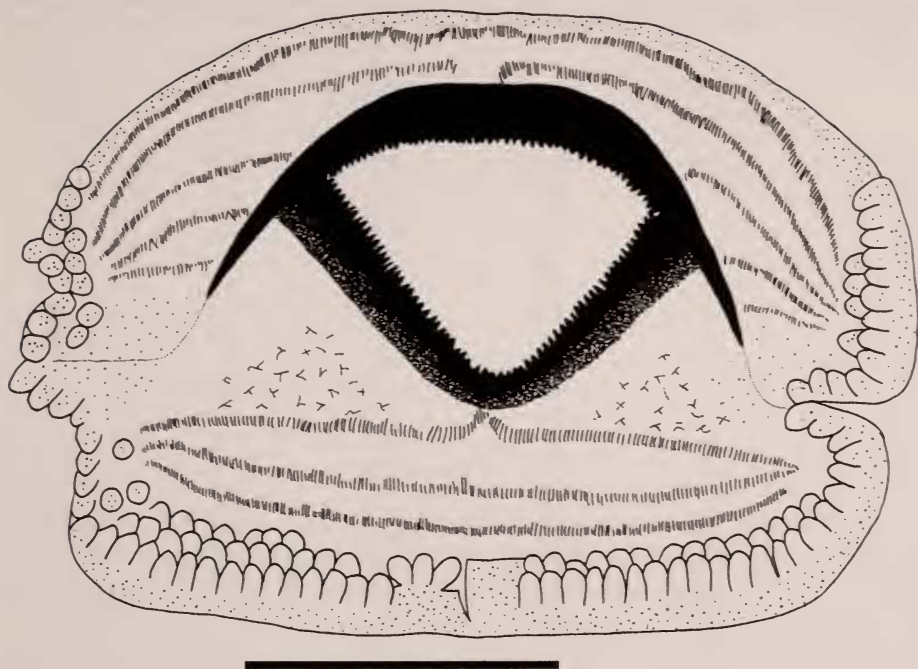


FIG. 5

Oral disc (scale bar = 1mm) of the preserved tadpole of *Rhacophorus maximus* (IEBR A.0942) at Gosner stage 35. Drawing by M. J. Wildenhues.

are green to violet dorsally, and fawn to yellow below. Webbings and discs are yellowish (Fig. 3C).

Body is elongated; head is wider than long (head length is 0.28 and head width 0.39 of total length); snout is rounded and slightly angular at the nostrils; eyes are very large (eye diameter is 0.58 of head length), pupil is round, with a dark line running from the eye towards the lateral end of the head; tympanum is not visible; nostrils are closer to the eye than to the tip of the snout; vomerine teeth and vocal sacs are indistinct.

Fore limbs are moderate (fore limb length is 0.62 of total length); the relative length of fingers is as follows: I < II < IV < III, with rounded to truncate discs, enlarged except for finger I; palmar tubercle and subarticular tubercles are present; webbing is present, webbing formula: II 1/2 - 1/2 III 1/2 - 1/2 IV.

Hind limbs are relatively long (length of hind limb is 1.2 of total length); the relative length of toes is as follows: I < II < III = V < IV, with rounded discs; inner metatarsal and subarticular tubercles are present; at the knee and at the tibio-tarsal joint there is a slightly pointed projection; toes are almost fully webbed except for toe IV.

Specimens ZFMK 89790 and MHNG 2721.52 (SVL 16.8-17.5 mm) largely correspond to this description (see Table 2 for measurements), as do other froglets that were only photographed at the Amphibian Station, except for nostrils being closer to

TABLE 1: Measurements (in mm) of the tadpoles of *Rhacophorus maximus* from the Tay Yen Tu Nature Reserve, Bac Giang Province, North-Eastern Vietnam; for abbreviations see material and methods.

	MHNG 2721.53	ZFMK 89791	IEBR A.0942	VNMM 1214	ZFMK 89792
stage	33	34/35	35	40/41	41
BH	5.2	5.2	5.4	5.9	6.4
BL	11.9	12.1	13.1	13.4	14
BW	5.6	5.5	6.7	8.6	8.4
ED	1.4	1.4	1.5	2	2.2
IP	4.1	4.1	4.3	5.2	5
IND	2.2	2.2	2.3	2.8	2.4
NPD	1.9	2.4	2.3	2.6	2.8
RND	1.9	1.8	1.5	1.9	1.6
SP	3.7	3.5	3.8	4.2	4
ODW	2.3	2.6	2.7	3.6	3
NK	38	37	35	42	-
NP	73	98	106	96	10.3
SS	7.5	7.5	8.7	8.6	9.2
TL	30.1	35.2	39.3	42.8	44.3
TAL	18.5	23.2	26.1	29.1	30.8
TMH	3.4	3.2	3.4	3.8	3.6
TMW	2.4	2.6	2.4	3.5	3
UF	1.6	1.4	1.5	2.3	1.5
LF	1.3	1.1	1.5	1.7	0.9
KRF	1:4+4/2+2:1	1:3+4/1+1:2	1:4+4/1+1:2	1:4+4/3	-
LTRF	5(2-5)/3(1-2)	5(2-5)/3(1)	5(2-5)/3(1)	5(2-5)/3	-

the tip of snout than to the eye.

Juveniles ($n = 4$; SVL 24.8-31.5 mm): The following description is based on specimen ZFMK 89788 (SVL 27.9 mm).

The juveniles in preservative differ from the available froglet series by having a more violet dorsum. The ventral side is white to fawn. Fore limbs and hind limbs are dorsally covered with violet pigments, with darker brown pigmentation on hands and feet, except for finger I. Venter is fawn. Colouration of dorsum fades to colouration of belly. Except for the region of the cloaca and the outer arm, starting from elbow until the tip of finger IV, and except for the region of the outer leg, starting from tibio-tarsal joint until the tip of toe V, where a white line followed by a brown line is separating the violet region from the ventral colouration.

In life, dorsum is light green. The green region fades to the light yellow venter with a narrow white stripe on the flank. Dorsal surface of fore limbs and hind limbs is light green with a lateral white stripe, more distinct on the hind limbs. Toes are dorsally grey to white with yellowish discs. A black spot is sometimes present on the throat (Fig. 3D).

Body is oval; head is large and long (head length is 0.32 and head width 0.41 of total length); snout is rounded, with distinct canthus rostralis; eyes are moderately large (eye diameter is 0.34 of head length); supratympanic fold is distinct; tympanum is very small (diameter of tympanum is 0.45 of diameter of eye); nostrils are equi-

Tab. 2: Measurements (in mm) of freshly metamorphosed froglets of *Rhacophorus maximus* from the Tay Yen Tu Nature Reserve, Bac Giang Province, North-Eastern Vietnam; for abbreviations see material and methods.

	MHNG 2721.52	ZFMK 89790	IEBR A.0941
stage	45	46	46
SVL	17.5	16.8	19.0
HW	6.6	6.7	7.4
HL	4.4	4.8	5.4
EN	1.4	1.5	1.6
NS	1.2	1.7	1.8
ED	2.7	2.8	3.1
TD	-	-	-
FLL	11.8	11.7	11.9
HLL	22.6	20.3	22.9
FL	6.7	6.2	7.4
IMT	0.9	0.8	1.1

distant from snout and eyes; vomerine teeth are distinct; vocal sac is not visible.

Fore limbs are relatively short (fore limb length is 0.55 of total length); the relative length of fingers is the same as described above for froglets.

Hind limbs are relatively long (length of hind limb is 1.33 of total length); the relative length of toes is the same as described for froglets; feet are fully webbed.

Specimens MHNG 2721.51 (except for the lacking tympanum and nostrils being closer to tip of snout than to eyes), VNMN 1212 (except for nostrils being closer to eyes than to tip of snout), and ZFMK 89787 (SVL 24.8-31.5 mm) largely correspond to this description (see Table 3 for measurements), as do other juveniles that were only photographed at the Amphibian Station in Hanoi.

Subadults ($n = 4$; SVL 38.4-50.8 mm): The following description is based on the wild-caught specimen IEBR A.0940 (SVL 50.8 mm), which proved to be genetically completely identical to the syntopic adult specimen IEBR 3653.

In preservative, the body is blue to violet with single white spots on the back. The light colour in the ventral region is clearly intercepted from the blue pattern on the back by a white stripe on the lateral side, followed by an irregular brown line, fading to the fawn belly. The white lateral stripe starts from behind the mouth to the groin. The upper jaw is lacking pigmentation at the mouth opening. Dorsal surface of the fore limbs, fingers III and IV are blue to violet, separated from the light colour on ventral side by a white stripe. Webbing, discs and fingers II-III are dorsally slightly brown. The ventral side of fore limbs is white to fawn, the same colour on dorsal fingers I and II. The dorsal surface of hind limbs, toes IV-V, and webbing between toes IV and V is blue to violet. The region around cloaca is brown pigmented with a short white stripe separating the blue from the brown colouration. Femur is fawn to yellow below, with brown pigmentation fading from the blue to the fawn region, lacking a white stripe. Femur has brown spots in the postlateral region. Tibia is fawn to yellow below. The knee region shows a white line anteriorly following a brown stripe separating the blue from the fawn region. Toes are fawn to yellow below. Webbing and discs have brown pigmen-

TAB. 3: Measurements (in mm) of juvenile *Rhacophorus maximus* from the Tay Yen Tu Nature Reserve, Bac Giang Province, North-Eastern Vietnam; for abbreviations see material and methods.

	VNMN 1212	MHNG 2721.51	ZFMK 89787	ZFMK 89788
SVL	27.5	24.8	31.5	27.9
HW	11.4	10.3	12.9	11.4
HL	9.2	8.3	9.7	9.0
EN	2.4	2.3	2.8	2.4
NS	2.6	2.1	2.9	2.5
ED	3.6	3.6	3.7	3.1
TD	1.9	-	1.4	1.4
FLL	16.0	12.7	16.3	15.2
HLL	35.5	31.7	43.9	37.0
FL	10.6	9.0	12.3	10.8
IMT	1.1	1.2	1.1	0.9

TAB. 4: Measurements (in mm) of subadult *Rhacophorus maximus* from the Tay Yen Tu Nature Reserve, Bac Giang Province, North-Eastern Vietnam; for abbreviations see material and methods.

	VNMN 1211	IEBR A.0940	MHNG 2721.50	ZFMK 89786
SVL	41.0	50.8	49.1	38.4
HW	15.9	19.6	19.1	14.4
HL	14.0	16.7	17.0	12.0
EN	3.7	4.3	3.9	2.9
NS	3.5	4.5	4.5	3.0
ED	5.0	5.6	6.0	5.1
TD	2.8	3.1	2.7	1.4
FLL	23.4	26.6	25.7	19.3
HLL	57.7	72.5	68.6	48.0
FL	15.8	21.3	20.1	13.5
IMT	1.5	2.2	1.5	1.5

tation dorsally. A white stripe followed by a brown stripe separates the blue from the fawn region posteriorly to tibio-tarsal joint, running down until the tip of toe V.

Dorsum in life is green with single light green spots on the back; venter is yellow to fawn. The light colour in ventral region is intercepted from the green colour on back by a white stripe on lateral side, followed by a brown stripe fading to the yellow belly. Dorsal surface of fore limbs, fingers III and IV are green. Lower surface of fore limbs is yellow with red fingers. The green colouration on the dorsal fore limbs is clearly separated from the white colour in ventral region by a white stripe. Fingers I and II are yellow on both upper and lower sides, including the discs. Webbing of fingers is completely red. Dorsal surface of hind limbs and fingers IV-V is green. The cloacal region is red to violet with a short white stripe separating the green from the red colouration. The inner edge and underside of femur are red. The red colouration of legs fades to the yellow to fawn belly. Tibia is red below. The anterior knee region shows a white stripe following a violet stripe separating the green from the red colour-

ration. Toes are red below, except for toe V being violet. Toes I and II as well as webbing between toes are red, except for the webbing between toes IV and V, being green in part. Toe III is red above. From tibio-tarsal joint downwards a white stripe followed by a narrow violet stripe is separating the green upper from the red lower (Fig. 6A, B).

Body is stocky, width at chest is double the width at hip. Head is strikingly large (head length is 0.33 and head width 0.39 of total length). Snout is rounded and slightly surpasses lower jaw. Canthus rostralis is distinct and angular. Nostrils are laterally positioned, roundish to triangular, and slightly closer to the eyes than to the tip of snout. Eyes are relatively large (eye diameter is 0.33 of head length). Tympanum is small (diameter of tympanum is 0.56 of eye diameter) and partially covered by a distinct supratympanic fold. Vomerine teeth are distinct. Tongue is deeply notched, vocal sac is indistinct. The dorsal skin is smooth. Belly and femur are covered with coarse granules below.

Fore limbs are rather small (length of fore limb is 0.53 of total length) with a relatively finger length as described for froglets and juveniles. Adhesive discs are enlarged and round to triangular, smallest on finger I. Fingers are nearly fully webbed. Subarticular tubercles are well developed and lumped. Palmar tubercle is well developed, whereas thenar tubercle is absent. Metacarpal tubercles are only indistinctly discernible.

Hind limbs are long and relatively slender (length of hind limb is 1.43 of total length). The relative length of toes is the same as described for froglets and juveniles. Adhesive discs are enlarged and round to semicircular, smallest on finger I. Toes are fully webbed. Subarticular tubercles are well developed and lumped. The inner metatarsal tubercle is rather flat and oval. The outer metatarsal tubercle is lacking.

The wild caught specimens MHNG 2721.50, VNMN 1211 and ZFMK 89786 from Tay Yen Tu with SVL 38.4-49.1 mm largely correspond to the above description, except for white spots on back and brown spots in the groin region (see Table 4 for measurements). However, subadult *R. maximus* ($n = 7$; SVL 37.8-61.0 mm) with the origin Tay Yen Tu bred in the Amphibian Station at Hanoi (and meanwhile kept in the amphibian unit of the Cologne Zoo) differ from the above description by lacking the distinct red webbing and the distinct red venter of hind limbs. In one subadult of *R. maximus* (SVL 53.8 mm) kept at the Cologne Zoo, we also observed that body portions such as the posterior and inner flanks, temporarily covered by the hind limbs, were slightly lighter (light green to yellow) than surrounding, not covered dorsal and lateral skin (Fig.6D).

DISCUSSION

The genus *Rhacophorus* Kuhl & Van Hasselt, 1822 contains approximately 74 species (Li *et al.*, 2008), and is widely distributed across India, China, Japan, mainland South-East Asia, the Greater Sunda Island and the Philippines (Frost, 2009). In general, tadpole descriptions are only known for 17 species of about 74 reported *Rhacophorus* representatives (Flower, 1896; van Kampen, 1907; Pope, 1931; Bourret, 1942; Alcalá & Brown, 1956; Inger, 1966; Iwasawa & Kawasaki, 1979; Inger, 1985; Inger & Tan, 1990; Hosoi *et al.*, 1995; Stuart *et al.*, 2006; Gillespie, 2007). Of the 18 *Rhacophorus*

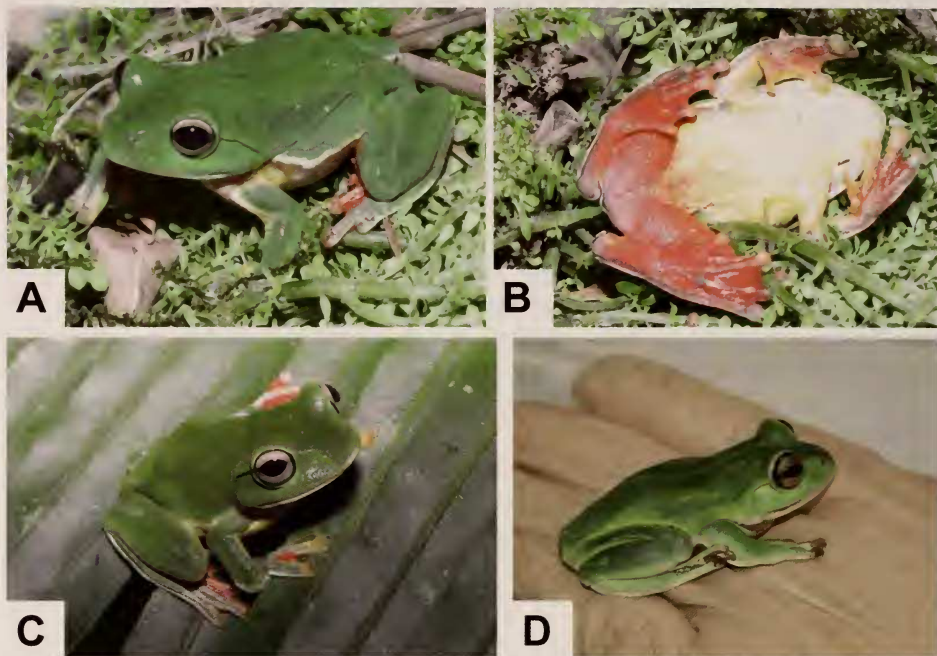


FIG. 6

Subadult *Rhacophorus maximus* (IEBR A.0940) from the Tay Yen Tu Nature Reserve, Bac Giang Province, north-eastern Vietnam, in life with distinct red webbing: (A) from above, (B) from below. Photographs by T. Ziegler. Subadult *R. maximus* in life: (C) in the habitat at Tay Yen Tu, and (D) in the terrarium at the Cologne Zoo. Photographs by T. Ziegler (C) and by D. Karbe (D).

species described from or recorded so far for Vietnam (Frost, 2009), only four larval descriptions are currently available, and among those only one tadpole description exists for Vietnamese *Rhacophorus* (see Table 5). However, tadpole descriptions are required because they facilitate identifications of early developmental stages of anurans and thus help to better understand their ecological requirements, which are important prerequisites for conservation measures.

Our tadpole description of *Rhacophorus maximus* from Vietnam largely agreed with larval descriptions available for other species of the genus in their main characteristics (Bourret, 1942; Inger, 1966): e.g., snout slightly rounded, eyes positioned laterally or dorsally; spiracle on left side; vent on right side (which does not reach the edge of the ventral fin); dorsal tail fin does not reach beyond base of tail. *Rhacophorus* larvae generally show a rather uniform morphology (Inger, 1966; 1985; Chou & Lin, 1997; Grosjean, 2004).

However, some species of *Rhacophorus* possess an uninterrupted row of marginal papillae on the lower labium, whereas others show a short medial gap (Hendrix *et al.*, 2007). With respect to the oral disk of the five *R. maximus* tadpoles studied by us, a medial gap in the lower lip is discernible. However, according to the tadpole

TAB. 5: List of Vietnamese *Rhacophorus* representatives (according to Frost 2009) including state of knowledge regarding morphological larval descriptions. * = Inger's (1985) larval description was based on *R. bimaculatus*.

<i>Rhacophorus</i>	larval morphology known (based on non-Vietnamese larvae)	larval morphology known (based on Vietnamese larvae)
<i>annamensis</i>		Hendrix <i>et al.</i> (2007)
<i>appendiculatus</i>	Inger (1966): Borneo	
<i>bipunctatus</i>		
<i>calcaeus</i>		
<i>chuyangsinensis</i>		
<i>dennysi</i>	Pope (1931), Bourret (1942): China	
<i>dorsoviridis</i>		
<i>duboisii</i>		
<i>dugritei</i>		Wildenhues <i>et al.</i> (in prep.)
<i>exechopygus</i>		in preparation
<i>feae</i>		Wildenhues <i>et al.</i> (in prep.)
<i>hoanglienensis</i>		
<i>hungfuensis</i>		
<i>kio</i>		Wildenhues <i>et al.</i> (in prep.)
<i>marmor dorsum</i>		
<i>maximus</i>		Wildenhues <i>et al.</i> (this paper)
<i>orlovi</i>		in preparation
<i>rhodopus</i>	Inger (1985): Borneo*	Wildenhues <i>et al.</i> (in prep.)

drawings of Anders & Rai (2002) *R. maximus* larvae from Nepal obviously have no medial gap in the lower lip. In addition, the keratodont formula of larvae from Nepal differs not only from that of tadpoles from Vietnam, but also does not agree with the general keratodont formula for the genus *Rhacophorus* given by Bourret (1942), i.e., 2:1+1/3 in *R. maximus* larvae from Nepal versus 4-7/3-4. Although we observed a slight variation within the keratodont formula of tadpoles of *R. maximus* from Northern Vietnam, differences were not such distinct as it is obvious from the illustrations of larvae from Nepal provided by Anders & Rai (2002). Maybe tadpoles of *R. maximus*, which were illustrated in Anders & Rai (2002) were in early developmental stages. This could explain the aforementioned differences, because only the number and arrangement of labial tooth rows subsequent to Gosner stages 25-26 are stable enough to be species-specific (Altig & McDiarmid, 1999). Earlier stages of tadpoles can be compared only with specimens in the same stage of development (Gawor *et al.*, 2009).

Moreover, we could show for the first time that subadult *Rhacophorus maximus* have strong red colouration on hands, feet and lower, inner and outer legs. This obvious colour pattern change within individual development first led us to the assumption of a new species discovery, but was discarded regarding the results of our genetic comparisons. The assumption that the colour pattern change in *R. maximus* is age dependent is underlined by the fact that red webbings only occur in wild-caught subadults from Vietnam but are lacking in syntopic adults. However, the lack of distinct red limb colouration in specimens from Vietnam kept at the Cologne Zoo, which at best show a slight, pale red pattern, shows at least that it may be subject to husbandry and/or climatic conditions. This, as well as the colour pattern change in the green ground colouration as described above certainly deserves further research. Among the

tree frogs occurring in the Indochinese region of South-East Asia, the following nominal species show red web on feet and green dorsal ground colour (Bordoloi *et al.*, 2007): *R. bipunctatus* Ahl, 1972; *R. htunwini* Wilkinson, Thin, Lwin & Shein, 2005; *R. kio* Ohler & Delmore, 2006; *R. malabaricus* Jerdon, 1870; *R. reinwardtii* Schlegel, 1840; *R. rhodopus* Liu & Hu, 1960; *R. suffry* Bordoloi, Bortamuli & Ohler, 2007, and *R. yaoshanensis* Li & Hu, 1962. In view of our results, the question must be raised whether the red webbing between toes and fingers of some of the latter species is just an age dependent colour pattern change, or whether red webbing may occur in certain developmental stages of additional *Rhacophorus* species, but was not yet recorded so far.

With respect to *Rhacophorus maximus*, further questions remain to be clarified: are the differences between Western and Eastern tadpoles due to developmental stages, as was discussed above, or does this already indicate possibly differing taxa? DNA fragments of *R. maximus* from Vietnam (IEBR 3653) compared to those of *R. maximus* from Simao, Yunnan, China (GenBank accession number: EF564548) were not completely identical (ca. 0.6% difference). Thus, a more in-depth genetic comparison of the more distant Eastern populations with Western populations would be worthwhile. Unfortunately, comparative material from Nepal could not be analysed so far. This is also underlined by the recently described *R. suffry* from India, which seems very similar to the red-webbed developmental stage of *R. maximus* from Vietnam as described in this paper.

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